

SEQUENCE LISTING

<110> INSTITUT PASTEUR  
CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

<120> Method for in vivo modification of the synthesis activity of a metabolite by modification of a gene the activity of which is not the original activity.

<130> BIF 023274 PCT

<140> PCT/US/FR03/xxxxx  
<141> 2003-03-28

<150> FR 03 03910  
<151> 2003-03-28

<160> 15

<170> Patentln version 3.1

<210> 1  
<211> 474  
<212> DNA  
<213> Lactobacillus leichmannii

<220>  
<221> misc\_feature  
<222> (1). (474)  
<223> Coding region of the N-deoxyribosyltransferase gene (dtp)

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<400> 1
atgccaaaaa agacgatcta cttcgggtgcc ggctggttca ctgaccgcca aaacaaagcc      60
tacaaggaag ccatggaagc cctcaaggaa aacccaacga ttgacctgga aaacagctac      120
gttcccctgg acaaccagta caagggtatc cgggttgatg aacaccgga atacctgcat      180
gacaagggtt gggctacggc cacctacaac aacgacttga acgggatcaa gaccaacgac      240
atcatgctgg gtgtctacat ccctgacgaa gaagacgtcg gcctgggcat ggaactgggt      300
tacgccttga gccaaaggcaa gtacgtcctt ttggtcatcc cggacgaaga ctacggcaag      360
ccgatcaacc tcatgagctg gggcgtcagc gacaacgtga tcaagatgag ccagctgaag      420
gacttcaact tcaacaagcc gcgcttcgac ttctacgaag gtgccgtata ctaa          474

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<210> 2  
<211> 157  
<212> PRT  
<213> Lactobacillus leichmannii

<220>  
<221> MISC\_FEATURE  
<222> (1)-(157)

<223> N-deoxyribosyltransferase carrying the mutation G9S.

<220>

<221> MISC\_FEATURE

<222>(9)..\_(9)

<223> serine/glycine mutation

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Met Pro Lys Lys Thr Ile Tyr Phe Ser Ala Gly Trp Phe Thr Asp Arg
1          5          10          15

Gln Asn Lys Ala Tyr Lys Glu Ala Met Glu Ala Leu Lys Glu Asn Pro
          20          25          30

Thr Ile Asp Leu Glu Asn Ser Tyr Val Pro Leu Asp Asn Gln Tyr Lys
          35          40          45

Gly Ile Art Val Asp Gly His Pro Gly Tyr Leu His Asp Lys Val Trp
          50          55          60

Ala Thr Ala Thr Tyr Asn Asn Asp Leu Asn Gly Ile Lys Thr Asn Asp
65          70          75          80

Ile Met Leu Gly Val Tyr Ile Pro Asp Glu Glu Asp Val Gly Leu Gly
          85          90          95

Met Glu Leu Gly Tyr Ala Leu Ser Gln Gly Lys Tyr Val Leu Leu Val
          100          105          110

Ile Pro Asp Gly Asp Tyr Gly Lys Pro Ile Asn Leu Met Ser Trp Gly
          115          120          125

Val Ser Asp Asn Val Ile Lys Met Ser Gln Leu Lys Asp Phe Asn Phe
          130          135          140

Asn Lys Pro Arg Phe Asp Phe Tyr Glu Gly Ala Val Tyr
145          150          155
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<210> 3

<211> 474

<212> DNA

<213> Lactobacillus leichmannii

<220>

<221> misc\_feature

<222> (1)..(474)  
<223> Coding sequence of mutated N-deosyribosyltransferase(NTD\*).

<400> 3  
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tacaaggaag ccatggaagc cctcaaggaa aacccaacga ttgacctgga aaacagctac 120  
gttcccctgg acaaccagta caagggtatc cgggttgatg aacacccgga atacctgcat 180  
gacaagggtt gggctacggc cacctacaac aacgacttga acgggatcaa gaccaacgac 240  
atcatgctgg gcgtctacat ccctgacgaa gaagacgtcg gcctgggcat ggaactgggt 300  
tacgccttga gccaaaggcaa gtacgtcctt ttggtcatcc cggacgaaga ctacggcaag 360  
ccgatcaacc tcatgagctg gggcgtcagc gacaacgtga tcaagatgag ccagctgaag 420  
gacttcaact tcaacaagcc gcgcttcgac ttctacgaag gtgccgtata ctaa 474

<210> 4  
<211> 32  
<212> DNA  
<213> artificial sequence  
  
<220>  
<221> primer  
<222> (1)..(32)  
<223> primer codBL for the amplification of the PyrC gene

<220>  
<221> misc\_feature  
<222> (1)..(1)  
<223> n is a nucleotide comprising a base A, T, C or G.

<220>  
<221> misc\_feature  
<222> (2) .. (2)  
<223> n is a nucleotide comprising a base A, T, C or G.

<220>  
<221> misc\_feature  
<222> (3) .. (3)  
<223> n is a nucleotide comprising a base A, T, C or G.

<400> 4

nnnccccgggc ttcttgctcg cttctcgttt gg 32

<210> 5  
<211> 29  
<212> DNA

<213> Artificial sequence

<220>

<221> primer

<222> (1) .. (29)

<223> primer cynTR for amplifying the pyrC gene.

<220>

<221> misc\_feature

<222> (1).7(1)

<223> n is a nucleotide comprising a base A, T, C or G.

<220>

<221> misc\_feature

<222> (2). (2)

<223> n is a nucleotide comprising a base A, T, C or G.

<400> 5

nnggatccgt ttgaccgtag cgggcgaac

29

<210> 6

<211> 29

<212> DNA

<213> Artificial sequence

<220>

<221> primer

<222> (1)..(29)

<223> Primer codBR allowing deletion of the CodA gene for the construction of the PAK9 strain.

<220>

<221> misc\_feature

<222> (1)..(29)

<223> n is a nucleotide comprising a base A, T, C or G.

<400> 6

ngaattctta ttcgacactg ttagcctcc

29

<210> 7

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<221> primer

<222> (1)..(27)

<223> Primer cynTL used in order to delete the CodA gene in the construction of the PAK9 strain.

<220>  
 <221> misc\_feature  
 <222> (1)..(1)  
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<400> 7  
 ngaattcacg actgggttac agcgagc

27

<210> 8  
 <211> 35  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <221> Primer  
 <222> (1)..(35)  
 <223> Primer ycEL used to amplify a DNA fragment of E.coli (M G1655) containing the pyrC gene.

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 <221> misc\_feature  
 <222> (1)..(1)  
 <223> n is a nucleotide comprising a base A, T, C or G.

<220>  
 <221> misc\_feature  
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 <223> n is a nucleotide comprising a base A, T, C or G.

<220>  
 <221> misc feature  
 <222> (3)-(3)  
 <223> n is a nucleotide comprising a base A, T, C or G.

<400> 8  
 nnncccgggg ccgacctgct ggcccactct gacgg

35

<210> 9  
 <211> 38  
 <212> DNA  
 <213> Lactobacillus leichmannii

<220>  
 <221> Primer  
 <222> (1)..(38)  
 <223> Primer dinR used to amplify a DNA fragment of E.coli (M G1655) containing the pyrC gene.

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<222> (1)..(1)  
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<220>  
<221> misc\_feature  
<222> (2)..(2)  
<223> n is a nucleotide comprising a base A, T, C or G.

<400> 9  
nnggatcccc cggcggcagc gcctacggaa ccgctgcc

38

<210> 10  
<211> 37  
<212> DNA  
<213> *Lacobacillus leichmannii*

<220>  
<221> Primer  
<222> (1)..(37)  
<223> Primer yceR used for the amplification of transforming plasmid DNA during the preparation of the PAK9 strain.

<220>  
<221> Primer  
<222> (1)..(37)  
<223> Primer yceR used for the amplification of transforming plasmid DNA during the preparation of the PAK9 strain.

<220>  
<221> misc\_feature  
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<223> Nucleotide comprising a base A, T, C or G.

<400> 10  
ngaattctta atcagtaaat ggaatgacaa ttctgcc

37

<210> 11  
<211> 34  
<212> DNA  
<213> *Lactobacillus leichmannii*

<220>  
<221> Primer  
<222> (1)..(34)  
<223> Primer dinL used for the amplification of transforming plasmid DNA during the preparation of the PAK9 strain.

<220>  
<221> misc\_feature  
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<223> Nucleotide comprising a base A, T, C or G.

<400> 11  
ngaattcaaa tcgtagcttc ctgttgatcat-tagc

34

<210> 12  
<211> 22  
<212> DNA  
<213> Artificial sequence

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<221> Primer  
<222> (1)..(22)  
<223> Primer FP23 for the amplification of the ntd gene.

<400> 12  
cgccagggtt ttcccagtcg cg

22

<210> 13  
<211> 23  
<212> DNA  
<213> Artificial sequence

<220>  
<221> Primer  
<222> (1)..(23)  
<223> Primer RP23 for the amplification of the ntd gene.

<400> 13  
agcggataac aatttcacac agg

23

<210> 14  
<211> 30  
<212> DNA  
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<220>  
<221> Primer  
<222> (1)..(30)  
<223> Primer for the amplification of the cloned ntd gene in pSU19 or its mutant.

<400> 14  
gatatacata tgccaaaaaa gacgatctac

30

<210> 15  
<211> 36  
<212> DNA  
<213> Artificial sequence

<220>  
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<222> (1)..(36)  
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<220>  
<221> misc\_feature  
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<223> n is a nucleotide comprising a base A, T, C or G.

<220>  
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<222> (2)..(2)  
<223> n is a nucleotide comprising a base A, T, C or G.

<400> 15  
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36